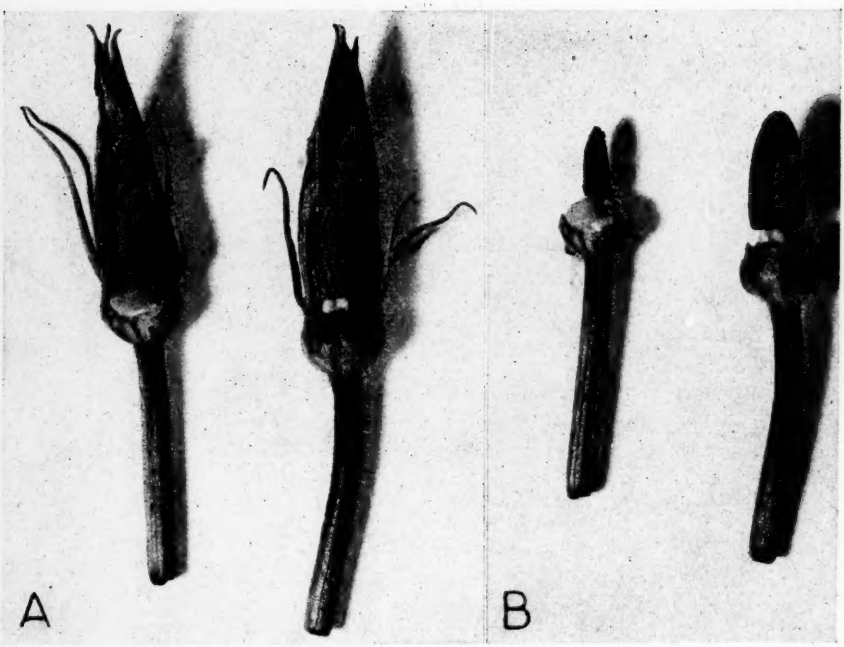
The Journal of HEREDITY



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"Broody" Roosters

Lint-Color Factors in Cotton

Variable Expression of the Ear-Pit Gene

Pedigree "Networks"—Eastwise or Westwise?

Inspiration versus Perspiration in Cytology

VOLUME 36 FEBRUARY, 1945 NUMBER 2

The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks

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The JOURNAL OF HEREDITY is published monthly by the AMERICAN GENETIC ASSOCIATION at 32nd Street and Elm Avenue, Baltimore, Md., and 1108 Sixteenth St., N. W., Washington, D. C. Address all communications regarding editorial matters to Room 205, 1108 Sixteenth St., N. W., Washington, D. C. Communications regarding the business management of THE JOURNAL OF HEREDITY may be addressed to the publication office, 32nd Street and Elm Avenue, Baltimore, Md., or to 1108 Sixteenth St., N. W., Washington 6, D. C. Entered as second-class matter February 24, 1923, at the postoffice at Baltimore, Maryland. Acceptance for mailing at the special rate of postage provided for in section 1103, Act of October 3, 1917, authorized December 29, 1918.

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The

Journal of Heredity

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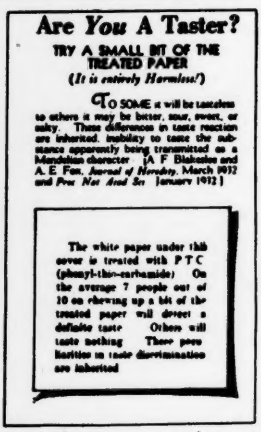
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LEAFLETS TO DEMONSTRATE HEREDITY

Last year the "Taster test" served as an introduction to human genetic differences in many classrooms. The test is so easily made with the treated paper, and arouses such great interest that it appears to have a definite place in group demonstrations of such differences. These leaflets, size 3⅜" by 5½", are available in quantities for those who wish to use them for classroom or lecture purposes. On the back is printed an outline 4-generation pedigree chart. Sample on request. Prices: 10 for 25c; 50 for $1.00.

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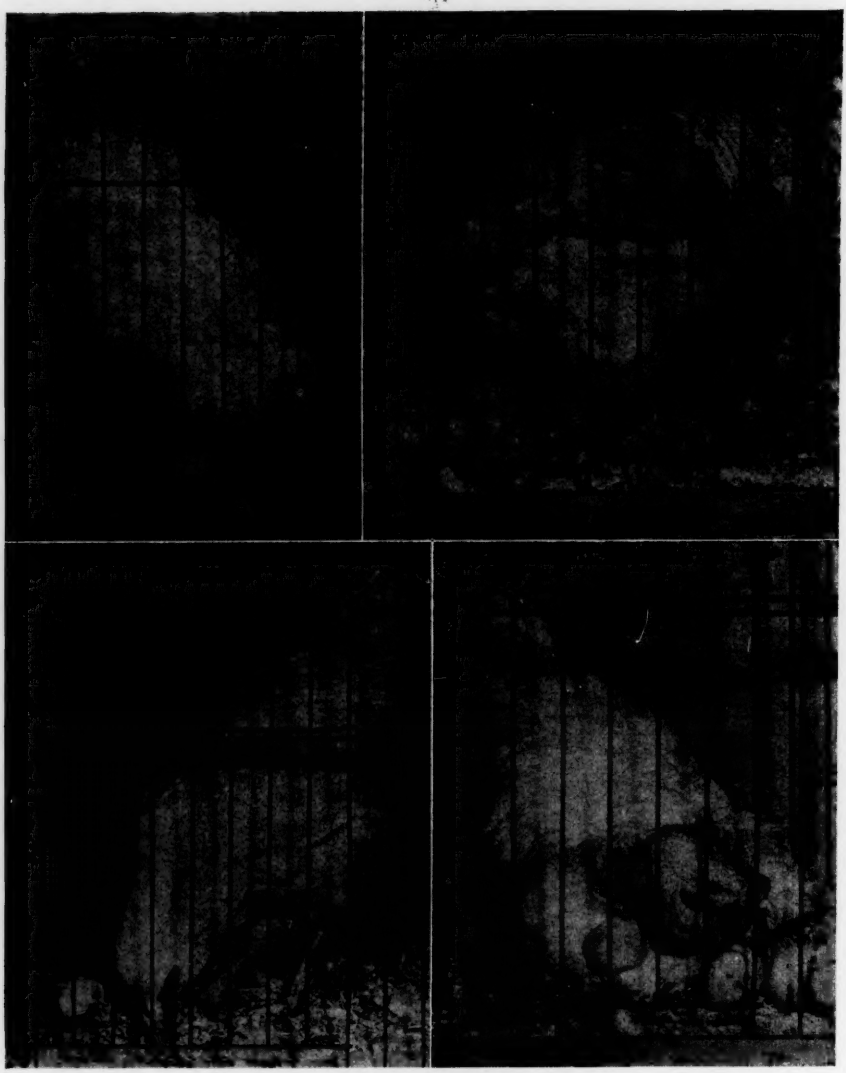


INVOLUNTARY "MOTHER"

Figure 1

Before prolactin injections this one-year-old Plymouth Rock rooster would ignore or kill any chicks placed in his coop. "Softened" by the maternal hormone he eagerly takes over the duties of a mother. These four views show him feeding and hovering a brood of twelve chicks. To give the full effect of this change, a sound-track would be necessary. Accompanying views *A* to *C* was a loud call similar to that heard when roosters attract hens, but more urgent and concerned. This "chow" call was accompanied by clucking, a sound never given forth by normal cocks.

ENDOCRINE IDENTIFICATION OF THE BROODY GENOTYPE OF COCKS

A. V. Nalbandov and L. E. Card
University of Illinois, Urbana, Illinois

Broodiness (the maternal instinct of chickens) is brought about by a pair of complementary autosomal genes[1] and in Cornish fowls in addition to the autosomal genes by a sex-linked gene.[2] Broodiness may be induced physiologically by injection of the pituitary lactogenic hormone (prolactin). Riddle and associates[3] believe that prolactin is effective only in those birds which are genetically conditioned to show broodiness. Capons can be caused to become broody after having been exposed to certain environmental conditions and to show at least part of the broody responses expected from a hen.[4]

Prior to the introduction of mechanical incubators and mechanical chick rearing devices it was of definite advantage to the poultry raiser to be in possession of "broody strains" because of their ability to incubate and raise the maximum number of eggs and chicks. Since the introduction of mechanical appliances and custom hatching, the maternal instinct has become not only superfluous but even disadvantageous. Broody hens do not lay, and require special handling during broody periods which last from three to twelve days and may occur repeatedly during spring and summer months. During recent years the tendency has been to eliminate this trait from domestic hens by rigorous selection against it. This has been complicated by the fact that the trait is not normally shown by males and since it is due to complementary genes two non-broody strains may, on crossing, produce a hybrid progeny having a high tendency toward broodiness.

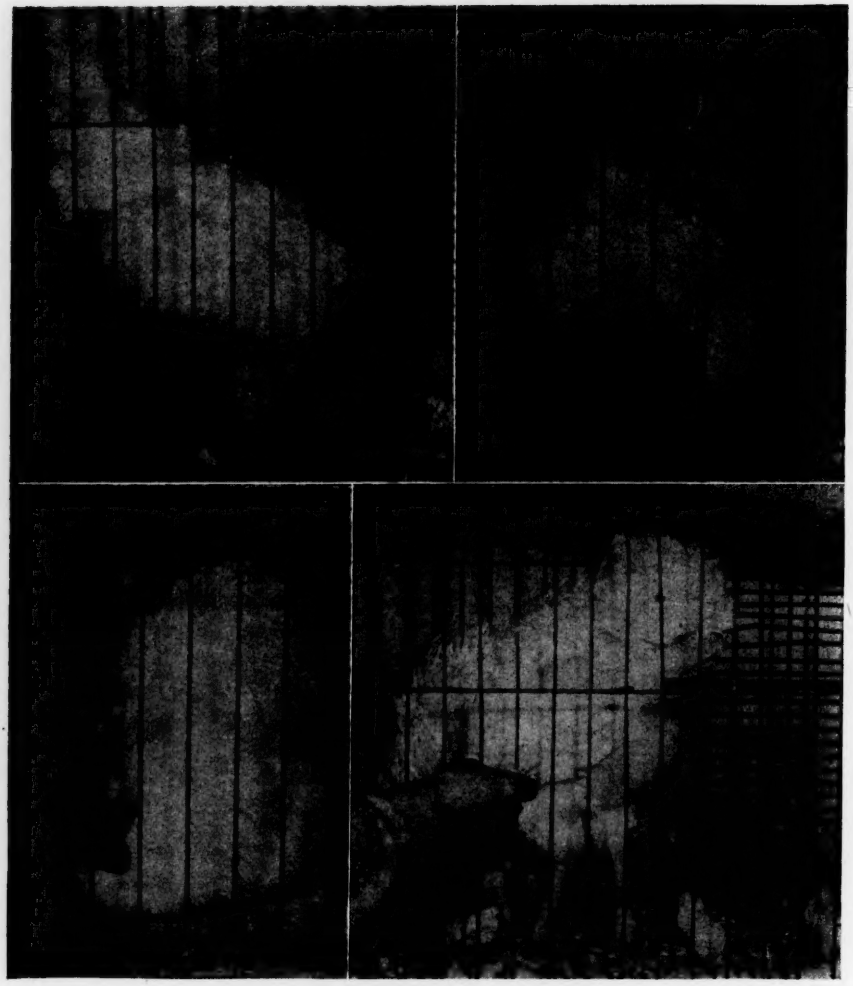
Theoretical Considerations

It is probable that the physiological function of the two or three genes for broodiness lies in their ability to regulate the rate at which the pituitary gland secretes prolactin. It has been shown by Burrows and Byerly[5] that the pituitaries from chickens belonging to broody strains are richer in prolactin than are the glands from non-broody strains. Presumably pituitaries of males secrete prolactin which, however, in the male is somehow prevented from producing its physiological effect. The fact that properly conditioned capons may be caused to become broody supports this assumption. Riddle's view that hens must carry either one or both of the complementary genes in order to become broody upon injection of prolactin was not borne out in preliminary trials in which small dosages of prolactin caused a complete broody response in hens both with and without a broody past. Furthermore, it was found that adult cocks treated with prolactin showed many phases of the maternal instinct just as intensely as it was shown by normally broody hens.

On the basis of the above results and considerations, it was reasoned that the amount of prolactin required to induce broodiness in the male should be proportional to the potential intensity of broodiness provided for by the genic makeup of males. Theoretically then, within a breed, the following relationship should exist between the genotype of the bird and the amount of hormone required to induce broody behavior:

Genotype	*Phenotype*	*Amount of Prolactin*
A—D	Intensely broody	+
A—d— and *a—D*	Broody or occasionally broody	++
a a d d	Not broody	+++

If this reasoning is correct it should be possible to differentiate between the various genotypes of males by hormone injection alone and without the expen-



HOVERING AND PROTECTING

Figure 2

A and *B* show hovering positions, in preparation for settling down for the night, accompanied by clucking and occasional hen-like singing, sounds never heard from normal males. Notice in *C* that the rooster has extended his wings and body fluff to give maximum warmth and protection. *D* shows a defensive position, accompanied by a shrill warning cry, obeyed by the chicks' beating a hasty retreat to the back of the cage.

sive and time-consuming breeding tests otherwise necessary. The experiments reported were set up to test these theoretical considerations.

Physiological Effects of Prolactin

Certain breeds of domestic chickens have greater tendency to exhibit broody behavior than do others. The White Leghorn breed was originally not as intensely broody as were other breeds and by continued negative selection the instinct for broodiness has been eliminated almost completely. Dark Cornish, on the other hand, are kept primarily for meat purposes and are both with regard to length and frequency of broody periods intensely broody. Taking these existing differences into consideration the first trials were conducted on males representing the White Leghorn, White Plymouth Rock, and the Dark Cornish breeds.

In the hen the broody instinct begins to operate as soon as a clutch of about 12 to 24 eggs is laid and consists of the following duties:

1. Sitting on eggs—this involves their warming and turning until chicks hatch.
2. Rearing the hatched brood—which involves the leading of chicks to feed and water, protecting and sheltering them under body and wing, attacking and driving away aggressors and a variety of vocal sounds, identified with most of the above tasks—and most of them peculiar only to the broody hen. Clucking is a typical example. A non-broody hen may be easily induced to show all of the above symptoms of broodiness following a single injection of prolactin.

Experiments with Males

I. The full expression of the broody instinct is enhanced by such purely environmental factors as warmth (about 90° F.), complete darkness and the presence of chicks.[6] Under these conditions hens and even immature pullets will become broody within 18 hours to 12 days after having been exposed to this environment. Accordingly it was decided to keep males in complete darkness immediately preceding the injection period and this was accomplished in a room held at 70-80° F. In no case did this treatment alone, even after exposure to chicks cause males to show any signs of broodiness.

In order to permit prolactin-treated males to express the effect of the hormone in a form more tangible and more measurable than clucking it was first attempted to cause a male to brood and hatch a clutch of eggs. For this experiment a male was placed in a small coop kept in complete darkness and with access to feed and water. The hormone was injected intramuscularly once daily. One hundred international units of prolactin (Armour)* were given per injection. Preliminary trials have shown that in any but the Cornish males a total of more than 300 I. U. is necessary to produce a response. On the third day of the experiment nesting material (straw) and one dozen eggs were made available to the male inside the coop. Regardless of breed and dosage of hormone none of the 12 males used was ever induced to build a nest.

In the second series of experiments a nest was built and filled with 12 eggs but even this inducement did not cause any of the males to sit on the nest. In fact, when a very small coop was provided in which most of the floor space was occupied by nest and eggs, the treated males preferred perching on the rim of the nest to sitting on the eggs.

During these trials it was observed that some of the injected males became very excited when small chicks were within hearing or seeing distance. When chicks were placed inside the coop, treated males immediately took charge of them and began active brooding operations. These observations led to the second phase of these experiments.

II. In these experiments males were allowed to manifest the hormone-induced instinct on chicks rather than eggs. This phase of the broody instinct includes the rather involved ritual of brooding the chicks under body and

*The Prolactin used in these studies was kindly furnished by Armour Laboratories of Chicago, Illinois.

wings, hovering, leading them to feed, tidbitting, calling them, and leading them away from danger by warning signals and protecting them from attacks by aggressors. Most of these events are accompanied by clucking sounds typical of the broody hen.

All subsequent tests were conducted in a coop measuring 8×6×4 feet. It was enclosed on all sides by cardboard causing the interior to be in semi-darkness. Several flaps cut in the sides and top of the coop permitted access to and observation of the interior. This coop was not heated and it was placed in a laboratory kept between 65 and 75° F. This temperature is 25 to 35° F. lower than required for optimum survival and thriving of day-old chicks.

Behavior of Untreated Males

A series of tests on uninjected males was undertaken. A male was placed in the coop with six day-old chicks. The response of untreated males towards the chicks differed individually. Some of the cocks retreated to the far corner of the coop and paid no attention to the chicks. When the chicks accidentally or purposely crowded around the male for warmth he would change position so as to escape from their proximity. This type of response was most frequently observed in White Plymouth Rock males. Most Leghorns and all Cornish males used, reacted to the presence of chicks by attacking them and killing them, usually by blows on the head. In no case did any of the untreated males show any of the broody responses toward chicks.

Behavior of Prolactin-Injected Males

As already mentioned, all males used in these experiments were kept in the dark prior to the beginning of injections. A male to be used in the experiment was removed from the dark room and placed in the experimental coop. Injections were begun on the same day. In all cases, regardless of breed of the male, chicks were placed with him on the second day of the experiment after he had received his second injection of 100 I. U. of prolactin. Invariably this small amount of hormone was not enough to cause a complete broody response and all of this first group of chicks would be dead or dying by the next day. A third injection would then follow and another group of chicks would be placed in the coop on the evening of the third experimental day. This procedure would be continued until the full broody response was obtained, i.e., until the chicks were adopted and cared for by the male. Unfortunately it was soon found that the complete broody response is not obtained suddenly but rather slowly so that for example, four of the second group of chicks may be killed, two of the third group; and it may not be until the fourth group that all the chicks would be accepted. This complicated the interpretation of results. It appears that the tolerance of males toward the chicks is gradually increased and is not an all-or-none reaction typically seen in the hen. The psychology of a male thus gradually approaching broodiness is somewhat difficult to understand.

Once the reaction is complete it parallels that of the hen to a remarkable extent. Males cluck, tidbit, and brood chicks under the wings by assuming a posture never observed in the normal male but typical of the broody female. Most of the males uttered the warning signal when their brood was threatened, retreated to the far end of the coop calling the chicks to follow (which they did) and circling back frequently urging stragglers to catch up. One White Plymouth Rock male invariably protected his chicks by flying into the observer's face with feet extended and feathers ruffled when an attempt was made to remove some of the brood from the coop. None of the other males showed this extreme in defensive action.

Broodiness would continue as long as prolactin was injected. Once the injections were stopped the symptoms disappeared within two to four days after the last injection. The males gradually lost interest in the brood which died of exposure to cold or were killed by the male by blows on the head.

Dosages Required for Broody Response

Complete broody response was obtained in each of three Cornish males with 300 I.U. of prolactin in each case. In two White Plymouth Rock males 400 I.U. and in another two, 500 I.U. of the hormones were required for complete response, while of four White Leghorn males one required 500 I.U. and three required 700 I.U. each to become intensely broody (Table 1). By contrast it may be mentioned here that 50 I.U. of prolactin were found invariably sufficient to cause broodiness in a non-broody hen of any breed.

Discussion

The primary reason for these experiments was the hope that it might be possible to determine the genotype of males by endocrine methods and without recourse to the expensive and time-consuming progeny test. If this method is to be of practical significance it must be applicable within a breed and its endpoint must be sufficiently sharp to eliminate the possibility of error in differentiating between genotypes. Unfortunately the criterion used in these trials (the behavior of injected males toward baby chicks) is not sufficiently sharp and clearcut to permit practical application. Complete broodiness in males is attained, or at least manifested, gradually and not suddenly as it is in females. Although the authors did not attain their practical objective, the interaction between genotype and "internal environment" was fascinating to observe, and we feel sure will interest many biologists and students of behavior. The accompanying photographs (Figures 1 and 2) give a faint insight into the very abnormal comportment of the treated males.

Of interest is the fact that regardless of the dosage of prolactin used no male was induced to incubate eggs. This points to the possibility that the presence of a second hormone is necessary to obtain the complete broody response including both incubation of eggs and care of chicks. It thus appears that the broody instinct of birds consists of two phases, the first of which—the building of a nest and the sitting on eggs—may be under the control of another hormone alone(possibly estrogen) or under the control of another hormone in conjunction with prolactin. On the other hand, the caring for the brood is quite clearly a prolactin effect.

The endocrinology of the action of prolactin in males will be discussed in another paper.

Summary

1. Regardless of amount of prolactin used males did not incubate eggs.

2. Males of the Cornish, White Plymouth Rock and White Leghorn breeds were made to brood and protect chicks. The amount of hormone required to cause this response is inversely proportional to the number of genes for broodiness assumed to be carried by the male. Untreated males invariably kill their brood or permit it to die through neglect.

Table I. Amounts of Prolactin Required to Produce Full Broody Response and the Hypothetical Genotypes of Breeds Involved

Breed	No. of males	Amount of Prolactin injected (I.U.)	Assumed genotype of males
Cornish	3	300	*AADDSS*
W. Plymouth Rock	2	400	*AADD—*
W. Plymouth Rock	2	500	*A—dd—*
S. C. White Leghorn	1	500	or *aaD—*
S. C. White Leghorn	3	700	*aadd—*

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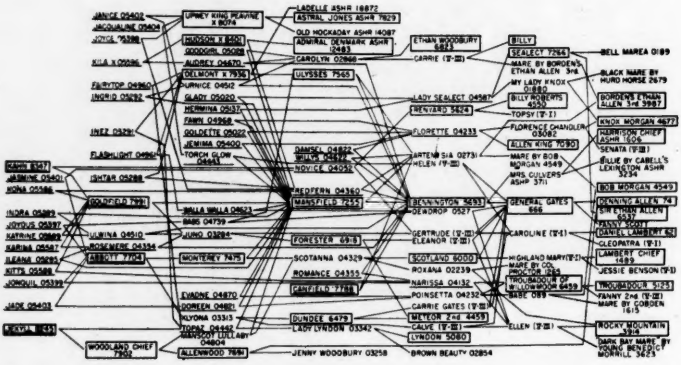
THE WRITING OF PEDIGREES



COMPLEX PEDIGREE

Figure 3

"Network" Pedigree including all the 49 animals in the U. S. Morgan Horse Farm in 1941. Underlined animals are in the stud at time of recording. Males are "boxed."

THE "genetiquette" of pedigree writing has been discussed in two recent issues of this Journal. Haines (*Jour. Hered.* 34:152) pointed out that, in designating crosses of animal breeds (for example, Landrace × Duroc Jersey), it is customary to list first the breed to which the male used in the cross belonged and the breed of the female second. This practice is not only customary, but is in agreement with the established practice of listing males above females in pedigree charts. In the interest of uniformity, both these practices should be generally adopted.

Hollander (*Jour. Hered.* 35:300) questions the use of separate pedigree charts for six individuals, supplemented by hieroglyphics, in a recent paper by Gregory, Mead and Regan (*Jour. Hered.* 35:195-200). His point with regard to this is well taken, and the "network" type of chart presented by Hollander certainly conveys a clearer picture of relationships among the animals than do six separate pedigrees. However, it is felt that the suggested "network" pedigree could be improved in two ways: (1) Introduce some method of distinguishing between sexes; and (2) Read from left to right in the style usually used in pedigrees.

The placing of males above females would necessarily be discarded in a "network" pedigree in many instances, but the sexes can easily be distinguished by enclosing males in "boxes."

When a pedigree is written in what Hollander calls "Oriental" or "right-left" style, it is usually read from the left, for the worker studying the pedigree is interested in an existing animal and uses the pedigree to trace its ancestry. Reversal of the pedigree, as suggested by Hollander, would actually result in reading in right to left fashion, in most cases, and would be confusing in calculating inbreeding coefficients. The "stripped" line pedigree in which only those animals involved in calculations are shown, and which is so convenient in determining these coefficients, would have to be composed and read from right to left, a very awkward procedure.

An example of how these principles can be applied to rather large groups of animals is shown in the accompanying figure from a paper by Phillips, Speelman and Williams (*Vt. Horse and Bridle Trail Bul.* 6:7-14, 1942). It shows

the ancestry of 49 animals that were in the stud at the United States Morgan Horse Farm, Middlebury, Vermont, when the pedigree chart was made.

In summary, the basic principles outlined are:

(1) Pedigrees should be written with the youngest individual at the left.

(2) "Network" pedigrees should be used whenever feasible to show the ancestry of related animals.

(3) In "network" pedigrees, males should be enclosed in rectangular boxes.

(4) In simple pedigrees males should be listed above females. This principle should be extended into "network" pedigrees when feasible.

(5) In specifying crosses between breeds (or genus or species) the male component should be listed first.

Adoption of these principles is recommended in the interest of uniformity and convenience.

RALPH W. PHILLIPS
U. S. Department of Agriculture, Beltsville, Md.

Comment by Dr. Hollander

Dr. Phillips' suggestion for a standardized form of pedigree network may be satisfactory for breeders whose interests are focussed on a few individuals and their coefficients of inbreeding and relationship. However, for population study and with plants or prolific species of animals the form which I previously suggested seems preferable. I would like to point out that the latter does show sex, and also sib groups and generations. Perhaps there is *Lebensraum* for two standardized methods, but I believe it is too early to crystalize only one.

W. F. HOLLANDER
Carnegie Institution of Washington

Clearly there are "networks" and "networks," suited in their different ways to organize various kinds of pedigree data. Very likely no standardized form, equally suitable for genealogizing peas, pigeons and percherons, will ever be evolved. Dr. Phillips' comments obviously apply only to livestock. Are there any other suggestions—a super-network, which works equally well "forwards" or "backwards," for breeding pens and for population? *Ed.*

PREPARING EARS OF MAIZE FOR GENETIC CLASSES

A MAJORITY of biological departments in our colleges and universities offer a laboratory course in genetics. Very frequently in these courses ripe ears of Indian corn are used as demonstration material. Ears on which the kernels are segregating for mendelian characters are excellent for acquainting students with the subject matter of genetics.

Many a teacher preparing for his demonstrations, has been disappointed when he opened the containers in which he had stored his corn, to find that beetles had riddled it with holes, that it had become either damp and moldy, or too dry so that the kernels had begun to fall off the ears. On three occasions it has been my duty to rescue large teaching collections of this sort and these experiences have yielded methods that I believe may prove of service to others.

The first collection had been stored in garbage cans. A dish of carbon tetrachloride had been placed at the bottom of each can to discourage beetles, and the lids had been fitted tightly. Someone prowling about the storeroom had removed the lids for no other reason apparently than to satisfy his curiosity. He had not replaced the lids. Beetles, mice and dry weather did the rest. From this collection of several hundred ears about fifty serviceable specimens with dropping kernels were rescued Good, heavy, clear, pre-war shellac was then

available. An attempt was made to brush the shellac on the ears but the grains were so loose that they came off in the process. Shellac was then poured into a cylinder and the ears were carefully dipped without touching the sides of the container. When dry, the grains were still too insecure to permit handling of the ears but they were attached firmly enough to allow brushing over with a second coat of heavy shellac.

The second collection was kept in boxes and was free from beetles, but the corn had become so dry that many of the grains were falling from the cobs and several otherwise excellent ears had been broken in two. This collection was being augmented by a new order which, when it arrived, was found to consist of old ears so dry that the slightest touch was enough to loosen the grains. Although each ear had been carefully wrapped in paper and meticulously packed for shipping, about 100 grains were shed in the individual wrappers of the shipment at the time of unpacking. Wartime shellac only was available. This was employed as in the first case, but this shellac was defective because the ears became sticky thereafter whenever it rained. It was found that the broken ears could be repaired readily by means of an internal cork core and Cascamite glue. It was desired to store the collection by hanging it on nails upon a wall and also to keep a record with each ear describing the type of cross that its segregation represented. Wartime scarcity prevented the purchase of screw eyes to fasten into the base of the cobs, but thin screw hooks were available for hanging the ears up separately. The hooks were bent into eyes by placing each hook in a vice and hammering its tip about a spike. Many cobs had burst and shrunk leaving a large hollow at the base and hence they provided no place for fastening the screw eyes. Such openings were stuffed loosely with paper toweling dipped in Cascamite glue. Before this had time to harden, the screw eye was turned into it and its base was daubed with Cascamite. The genetic data were typed upon a medium sized shipping tag that was tied to the screw eye. (Cascamite glue has the advantage of becoming very hard, of shrinking very little on drying, and of not becoming soft or sticky thereafter during wet weather.)

The third collection had been stored in glass Mason jars. Insect pests had been warded off by placing a large wad of chloroform-soaked cotton in the mouth of each jar before sealing. Hence the ears were constantly in moisture. The grains adhered to the cobs but on many ears some grains had become discolored and mushy. Upon handling, the mushy grains would fall to pieces. These ears were dried thoroughly before attempting to preserve them. My unfortunate experience with wartime shellac suggested that some other transparent substance should be used to glue the grains to the cob. A perfect solution to the problem was found in Plasti-Kote, a clear, plastic paint. Because Cascamite had been so successful previously, it was used again with cork stoppers for plugging cobs in order to permit the insertion of suitable screw eyes. To the screw eyes were attached shipping tags bearing the genetic data as previously. This collection is now stored on a wall where it has a chance to dry out, because it has to withstand a rainy season during which moisture would have been expected to penetrate the ears.

To summarize, I find the most successful method of preparation for ripe ears of Indian corn used in genetics classes to be: (1) a coat of hard, transparent plastic paint (Plasti-Kote) to glue the grains permanently to each other and to the cob, which procedure will also discourage insect pests, (2) the repairing of broken or hollow cobs with cork stoppers dipped in Cascamite glue, (3) the insertion of screw eyes in the base of cobs, (4) the attachment to the screw eyes of small shipping tags bearing pertinent genetic data, (5) the storing of the ears by hanging them on a wall in the open class room where they may remain dry and well aired.

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CONTRASTS IN CYTOLOGY

A Review

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SCHRADER'S new book,[1] *Mitosis,* brings into sharp relief the divergences in method and attitude which have developed in cytology and cytogenetics during the past fifteen years. He leads a school of cytology that stresses the comparative method, utilizing differences between natural species, and practices rigid induction from the most careful microscopic observations; his choice of materials, being so wide-ranging, usually gives little scope for correlated genetic experimentation. At the opposite extreme is the school of cytology led by Darlington, which stresses deduction, particularly from genetic data, and asserts that in case of conflict greater reliance should be placed on logical theory than on direct microscopic observations. Mutant or "abnormal" types furnish the materials for most of this school's comparative work.

Most cytogeneticists outside the Darlington school, particularly the maize and Drosophila workers, are Baconians, insisting on the importance of data, then on induction from the data. The function of deduction to them is to suggest new experiments or tests, not to build up general theories. They strive always for the most complete experimental correlation of genetics and cytology. Even those who accept Darlington's conclusions uncritically do not accept the tenets of his working principles for their own researches.

These divergences are in part similar to but more serious than the one which has developed in astrophysics between "Galileans" and "Neo-Aristotelians."[2*] More serious: (a) because not as clear-cut, (b) because the fundamental nature of the conflict has been obscured by clashes of personalities and is therefore not yet clearly recognized in cytology—the writer acknowledges with regret his own contribution to this—and (c) because while the astrophysicist can check his deduction with objective "pointer-readings" (or hopes ultimately to do so) the deductive cytologist's check is a highly subjective "observation" through the microscope—he is certainly very apt "to find what he is looking for."

Attempting a review of Schrader's book, the present writer found himself exploring the causes of its scientific humility, amounting almost to disillusionment, when compared with any other major treatise in cytology and particularly its astonishing contrast in all aspects with that from which it differs most — Darlington's *Recent Advances in Cytology.*[3,4] Perhaps his musings will aid other readers to find their own basis for the reconciliation of many views, and even data, that at first appear flatly contradictory; this not only in these two books but in numerous research papers from different schools of cytology, of which these authors represent the most diverse.

Beginnings of Wisdom

Schrader's title is "Mitosis," but he includes meiotic mitosis, now so generally given semi-independent status as "meiosis." In his Introduction he says, "If a dispassionate discussion of the subject of mitosis is possible, it is perhaps chiefly due to the fact that our failure to solve most of its problems is so manifest. With rare exceptions we are filled with proper humility—the humility of

*Evidently it occurs also in fields of biology other than cytogenetics. Since the above was in press, Edgar Anderson, in the November 1944 issue of this JOURNAL, cites Stanley A. Cain as echoing the opinion of H. M. Raup that "biogeography and ecology carry 'a heavy burden of hypothesis and assumption which has resulted from an overemployment of deductive reasoning. What is most needed in these fields is a complete return to inductive reasoning.'"

the open mind." Then he begins his analysis of mitosis with the structure of the living cell and compares it with the fixed cell. The components of the various types of spindles are summed up as: 1, "Continuous fibre"; 2, "Chromosomal fibre"; 3, "Interzonal connection"; 4, "Center"; 5, "Chromosome." The chromosome is "clearly a complex structure some or all of whose parts may actively participate in the mitotic process." There are "surprising gaps in our knowledge" of its structure. A "tentative" description of it involves consideration of the following parts: a, "'Genonema' (or chromonema)"; b, "Matrix . . . composed of at least two different substances"; c, "'Pellicle' (or sheath)"; d, "'Kinetochore' (or centromere)." The arguments for and against the actuality of structural elements in the spindle are fairly presented and at the end the personal conclusion boldly stated that "the reality of spindle fibres may be regarded as settled." The kinetochore or centromere is exhaustively yet briefly treated. It should almost satisfy the geneticist, the descriptive cytologist and the evolutionist, as may also the discussion of the aster and spindle components.

Hypotheses of the mechanism of mitosis occupy 36 pages and result in the conclusion that not one of them is satisfactory but that we *can* say: "The chromosomes themselves play an active part in the mitotic mechanism. Any movement beyond the initial separation is, however, associated with some part of the mitotic apparatus." A secure but very limited statement! The chapter on Related Problems covers the resting stage, pairing, the bouquet stage, telomeres, contraction phases, heteropycnotic attraction, kinetochore attraction, the nuclear membrane, and prometaphase stretch.

The general comment on these epitomises Schrader's general attitude: "The foregoing survey of problems that at present have no satisfactory answer will serve to show the range of the difficulties before us. . . . Above all they reveal the lack of completeness in our information concerning the properties of the nuclear membrane, the kinetochore, and finally the detailed structure and reactions of the chromosome. It is therefore not astonishing that attempts to connect the findings of physical chemistry with the biologist's knowledge of mitotic structures have progressed so little. Perforce such attempts are often no more than shots in the dark."

Again in the concluding chapter Schrader reiterates: "Not one of the many hypotheses that have been broached has in it the definite promise of a final solution." But he is not thereby daunted! He concludes that we have every reason to doubt that "the answer will ever be revealed by a stroke of genius or by luck. . . . We must make up our minds that we are confronted by some painstaking work that has to be guided by intelligent planning and a thorough knowledge of the results so far obtained." Schrader suggests, finally, that attacks should be made on a single limited phase of the mitotic cycle, not on the wider goals of the past. "This first step," he concludes, "will naturally be the most difficult of all." Succeeding phases "will become progressively easier." And so he concludes (as does the reviewer from a slightly different basis) that we are at the hopeful stage in cytology where, disillusioned by the failure of early hopes of easy success, we have fallen back, taken stock of our situation, replenished both our matériel and our morale and are ready to rise once again to the challenge.

Exaltation versus Skepticism

To those who have been exalted and carried away by the brilliance and success of Darlington's approach to cytological problems and have not yet seen its flaws, Schrader's book will appear needlessly depressing, dull and lacking in imagination. What a contrast to *Recent Advances in Cytology*. (That the Title and Foreword of Darlington's book, for neither of which he can be held responsible, are entirely in keeping with the Preface and Contents, emphasizes we must contrast schools of thought and method rather than individuals as such.

The Foreword (by J. B. S. Haldane) to the first edition of 1932, sets the keynote of youthful enthusiasm. The book "marks the beginning of a new epoch." Its "principles stand the test of practical application." "The last Chapter is a prolegomenon to every future theory of evolution"—though Haldane admits that he has "not yet fully digested it." Darlington in his Preface begins modestly by stating that he "attempts to describe one aspect of cytology." He warns the student that "he cannot learn the subject from 'bookish theoretic,' but he will find the first eight chapters a guide to his early observations." He promises in assimilating others' observations "to be candid but not impartial"—surely a fair enough warning. From a sound philosophical basis he discusses "fact" and "hypothesis" and emphasizes that the distinction between them as "made in every day life becomes too naive when applied to the data of a new study." He quotes to good effect the words of Huxley: "Those who refuse to go beyond the fact rarely get as far as the fact." Here again, if they will but heed it, he is warning his readers of his intention to deal with data as his own reason or intuition may dictate. He concludes his Preface by hoping that "If some of the hypotheses here advanced prove to be will o' the wisps, others . . . will lead to tangible discoveries."—the proper purpose of hypotheses! The book is, or should be, so well known to every cytologist and geneticist that an apology would be necessary for quoting from it were it not that most of his followers (on either side of the Atlantic) seem never to have read the Preface—or not as the warning the then youthful author intended it to be regarding the methods of his approach. That stimulating book led to many discoveries and contributed much to the wave which swept all cytology out of the backwater so much of it had lain in since 1900.

The 1937 edition, alas, reveals one of the prices that Success, "The Bitch Goddess," exacts. In the Foreword we find the author now elevated to unassailable heights by the simple but erroneous statement that most of the objections to the hypotheses of the first edition "have been quietly withdrawn in the four succeeding years." Credit is given or implied for discovery where it should really be for able and striking exposition of the too modestly and much too briefly announced earlier discoveries of John Belling and others. Further we are told that "General principles have been discovered of such wide validity that we can predict from them with considerable confidence, and on the rare occasions when the prediction is falsified, we are inclined to look for undetected causal agencies rather than to recast our first principles." This attitude, we are reminded, "has long been normal in chemistry and physics." The comparison is perhaps deceptive. To the writer the parallel is, in the present state of cytology, closer to theology. The Preface reveals the same changes in the author. In it we now learn that "I have shown mitosis as giving rise to meiosis . . . I have deduced the conditions of parthenogenesis from experimental observations of the breakdown of sexual reproduction" . . . "Ten years ago . . . we could scarcely see the wood for the trees" (True!) "Now order is beginning to appear. Causal relationships are being established." Finally: "Thus in the cell, mechanics, physiology and physical chemistry are being brought together to show the unity of living processes." Reading that and Schrader's final conclusion that we have yet to take the definitive first step towards such unification, can the non-specialist in cytology or the young student be other than mystified; could a reviewer do other than attempt an estimate of the reasons for the difference or give his own appraisal of their comparative merits?

Poisons and Antidotes

To which author should the young researcher turn for guidance? This writer says: if to either, then to both, or rather to both the schools of thought they represent, for the difference as emphasized earlier is in the end one of schools rather than of individuals. Darlington is

stimulating* ("over-stimulating" wrote John Belling). His hypotheses do lead to discoveries. But he does usually state them as if they were facts, (as his disciples[1] almost always do); does, as he warned he would, interpret data to fit his hypotheses; does work deductively from genetic facts to cytological observations, which are so often subjective, and prides himself on it. Are these demerits? Not necessarily. They are dangers and pitfalls for the unwary beginner, for the worker in allied fields, for the descriptive cytologist who is not experimentally or critically-minded. To the experienced worker, able readily to distinguish fact from phantasy, the book is invaluable. But one young research cytologist who keeps a copy of *Recent Advances* always handy on his desk has hit the nail on the head by labelling it "Poison for Students." Most scientists exercise the function of critic for their readers before they publish. (Schrader almost to excess!) Darlington leaves critical analysis to the reader. He continuously publishes guesses — and very often guesses right! Unfortunately he rarely acknowledges it when he guesses wrong and those who best could do so (instance those of the "maize group") rarely choose to point out his errors. Today there are not too many cytologists (and fewer geneticists) who know in what respect Darlington's hypotheses are still valid and useful and in what disproved. Schrader does! His book is therefore the current antidote, a "must" for every reader of Darlington.

Does Schrader provide the jumping off platform for that first attack he sees as so necessary? Yes! The student of Schrader will certainly know what we don't know in cytology, and that, we are told, "is the beginning of wisdom." He will know, further, what hypotheses have been proposed and what their present status is. Some that Schrader evidently doesn't like are neverthless clearly shown still to have possibilities in them or to need reconsideration rather than outright rejection. Those that have been invalidated are given what is, one hopes, the *coup de grace*.

Does Schrader provide the stimulus which will cause anyone to want to jump off his platform? Doubtful! The courage and dauntless determination which drives Schrader on is the determination that holds the line, consolidates positions, wins counterattacks, but does not usually lead to new firm steps differing in kind. That demands a naiveté that very, very rarely survives early youth in an intelligent man. Can Darlington make the confession of humility that Schrader has done and begin again? His admirers who are not his disciples will every one hope so. If not, then perhaps some younger worker trained (or well-read) in both schools will arise to take up the challenge. (There are, presently, encouraging signs!) He will probably find it better to start with Schrader. Or better yet with Sharp,[5] provided he doesn't stop there. And that applies to any reader!

Incidentally, the writer thinks our knowledge of mitosis is neither so limited as Schrader's debunking may make it seem nor so secure or extensive as Darlington's disciples imagine.

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3. Darlington, C. D. *Recent Advances in Cytology*. London and Philadelphia. 1932.
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*The deductive, "Neo-Aristotelian," method is in itself stimulating to certain types of mind. One of its adherents in the field of physics thus contrasts it with the inductive "Galilean" method:

"The genius of every age turns mainly in that direction which offers the greatest scope for the creative imagination. . . . A science kept strictly to facts and formulae by 'pale Galileans' [sic] would recruit very few geniuses, fewer rank and file workers, and—fewer endowments."—D. H. Stafford Hatfield, *Nature* 139:1089.

The writer is proud to disagree with the second part of this statement as a generalization, but is forced to acknowledge its truth in some cases—hence in part his present analysis of the effects of the different methods in cytology.

MALE STERILITIES AND ALBINO SEEDLINGS IN CUCURBITS

A Study in Inbreeding

OVED SHIFRISS*

FOR the past few years the writer has had the opportunity of observing large inbred populations of the cultivated cucurbits. Although no critical analysis of the genetical composition of these populations has been possible, certain distinct abnormalities have been observed. Among the latter were male sterilities, albino seedlings, and one intergeneric parallel variation of abnormal stem enlargement. It is the purpose of the present paper to report and evaluate some of these abnormalities.

Materials and Methods

Many commercial varieties of *Cucurbita pepo* L., *C. maxima* Duch., *C. moschata* Duch., *Cucumis sativus* L. and *Cucumis melo* L. were inbred for one to eight generations. The original commercial varieties were grown in most cases in the vegetable trials. Each trial row conisisted of 25 to 30 individuals and each variety was represented by one or two rows. Only Golden Delicious, a variety of *Cucurbita maxima* Duch., was planted on a larger scale one sample comprising at least 1,000 individuals.

Altogether a total of 2,000 self-pollinated fruits was obtained from all varieties during the first season of 1942 but only certain fruit types were saved. Therefore, the data presented in this report do not pertain to random sampling of inbred lines.

Strips of paperboard, 3×1 inches in size, were used in pollinating flowers of the genus Cucurbita. Pocket Tot (hand stapler) or paper fasteners were employed to fasten the two ends of the paper strips which were folded and between which the corolla was clasped. Glassine bags 2¼×3½ inches in size were used to protect the pollinated flowers. The bags were fastened also by paper fasteners or Pocket Tot, the latter being the more efficient method. The technique of tying the corolla tips by strings[5] or raffia before and after pollination was not proved to be efficient and safe with all varieties and therefore was used only to a limited extent.

In pollinating flowers of the genus *Cucumis* the corollas were fastened with OK paper fasteners the afternoon before anthesis. The next morning each pistillate flower was pollinated and its corolla was covered with a No. 000 gelatine capsule to prevent insect pollination. This method is used at the Department of Plant Breeding, Cornell University, Ithaca, N. Y., for pollinating cucumbers and melons and has been described by Munger.[11] (See Figure 4.)

The corollas of both pistillate and staminate flower buds were fastened the day before pollination. The former were marked by garden stakes with red tips placed between the rows but close to the vine on which the "clipped" pistillate flower was borne. This method enabled the pollinator the next morning to find these flowers without any difficulty. Pollinations were made in the morning between the hours of six and ten or eleven o'clock, depending on the temperature.

Results and Discussion

1. Incidence of Male Sterilities

Sterility in the cucurbits may be attributed to various circumstances such as *1. failure of syngamy* due to interspecific intergeneric,[17] and autopolyploid[15] cross-

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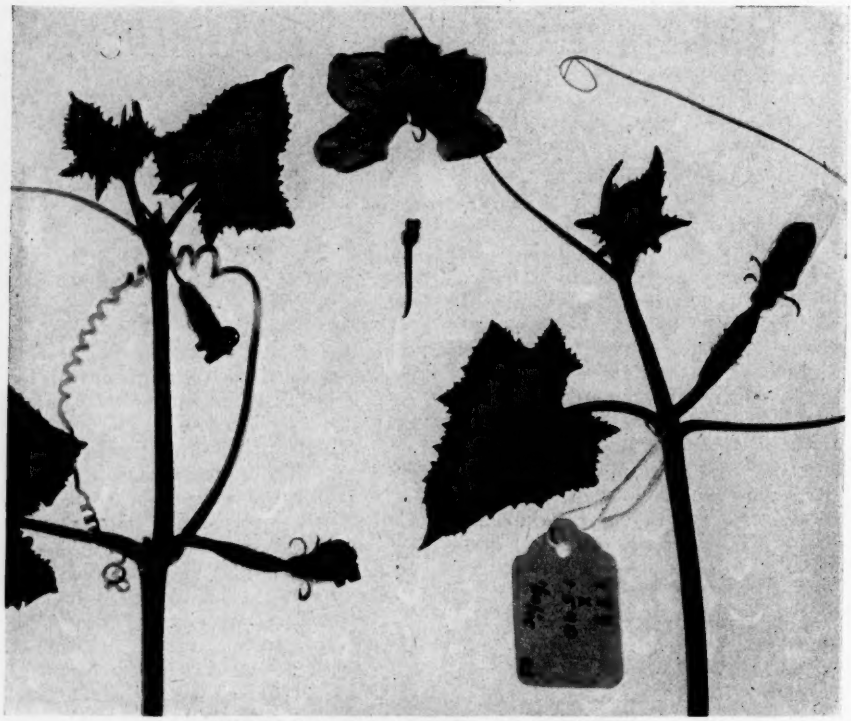


TECHNIQUE OF CUCUMBER POLLINATION

Figure 4

Left: Staminate and pistillate flowers are fastened with O. K. paper fasteners (Oakville Co., Waterbury, Conn.) the day before anthesis. Next morning the fasteners are removed and staminate flower detached from the plant and corolla removed (center). *Right:* A pistillate flower after pollination. Note the gelatine capsule (No. 000, Eli Lilly & Co., Indianapolis) which protects the pollinated flower from foreign pollen. Capsules eventually get soft and fall off. Photographs courtesy of W. Atlee Burpee Co.

es as well as to the detrimental effect of low and high temperatures on viability of pollen grains. Under such conditions fruit may develop as a result of pollen hormone stimulation but no good seed will set; *2. Ephemeral period of reproductive inactivity* due to ecological factors. Certain varieties, *Cucurbita pepo* L. and *Cucurbita maxima* Duch., when grown in the greenhouse during the winter months in Doylestown, Pennsylvania, did not produce seed because of abnormal sexual development of their staminate flowers. The latter were either deficient of the entire androecium or the anthers were completely aborted. The same varieties set seed abundantly in the field during the summer; *3. Genetical sterilities* which may be manifested in various ways and which are of special concern here.

Sinnott and Durham[16] state that a number of inbreds of *Cucurbita pepo* L. "refused to set seed when selfed" and one or two inbreds were lost subsequently. They also contended that "sterility or lack of vigor and not seasonal or other environmental differences were the causes of the failure." The writer found inbred lines of *Cucurbita pepo* L. which varied consistently in the amount of available pollen grains per staminate flower. Some inbreds had relatively little pollen while others had plump an-

thers and therefore were highly fertile. The former group did produce occasionally abundance of seed but this ability was not consistent.

Jakimovič[8] reported a decrease in number of seed per fruit in certain inbred lines of *Cucumis sativus* L. This finding is not in agreement with the contention of other workers but the writer does not know of any reliable data on the subject other than that of Jakimovič.

In addition to genetical sterilities resulting from "loss of vigor" there are a variety of morphological sterilities.

Hutchins[6] reported a green flowered variation in *Cucumis sativus* L. which was found to be a female sterile due to abnormalities of the pistillate flower. The same author[7] has recently described an interesting male and female sterile variant in *Cucurbita maxima* Duch.

Among 200 one-generation inbreds of *Cucurbita pepo* L. the writer found three lines which segregated for male sterile plants. These inbreds originated in three different commercial varieties. The total number of plants in each of the original inbreds was 61, 35, and 30 and the number of male sterile individuals was 14, 6, and 7 correspondingly. Additional data from F_1, F_2, and backcross populations indicate beyond any doubt that male sterility in these cases is inherited as a recessive character whose expression is due to a single gene difference. This morphological male sterility is expressed in complete abortion of the androecium at the bud stage before the staminate flowers open (see Figure 5). No such characteristic has been previously reported in the cucurbits.

Practical Significance of Morphological Male Sterility

One of the interesting recent developments in applied genetics is the realization of the possible value of male sterility in the production of hybrid seed in vegetable crops. There are numerous publications reporting the occurrence of male sterility in plants and in addition much unpublished data also exist. However, most of the reported male sterilities are not of immediate practical significance. An ingenious method whereby a cytoplastmic male sterility was utilized for hybrid seed production in *Allium cepa* L. has been reported by Jones and Clarke[10] of the United States Department of Agriculture.

The genetical male sterility reported here in *Cucurbita pepo* L. is also of considerable practical value. The maintenance and increase of this male sterility is possible by a propagation of the backcross population. This can be accomplished by staking all male sterile plants in the backcross population and by harvesting the seed from the same. Bees and other insects are the pollinating agents. Preliminary data indicate that twenty fruits harvested by this method may yield as much as one pound of seed or more.

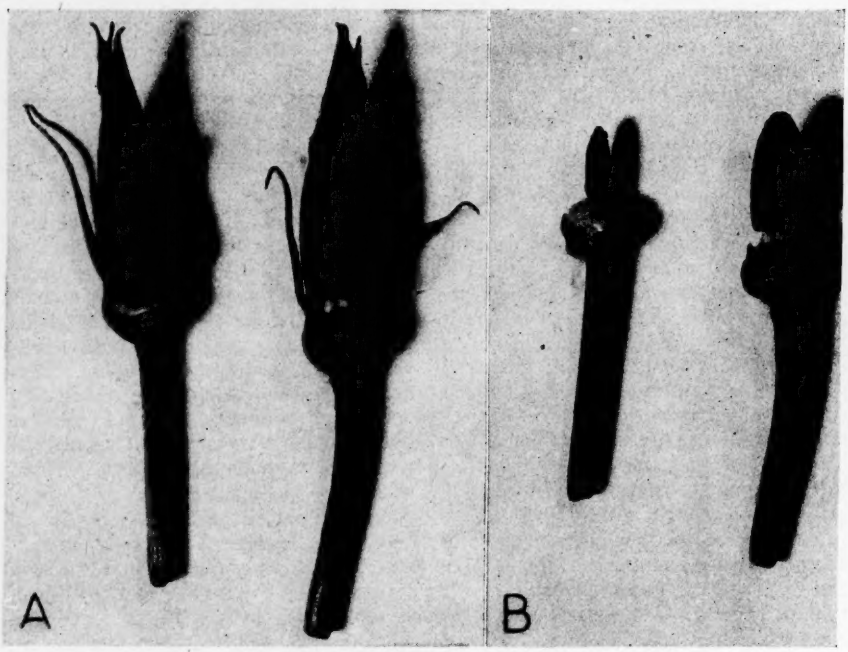
In commercial production of hybrid seed the "female" and "male" parent should be planted in alternate rows in ratios 3:1 or 4:1. The "female" parent is represented by the backcross population and all male fertile plants are removed during one roguing operation. The rate of seeding of the segregating backcross parent can be regulated so that at least one male sterile is present every three or four feet in the row. The roguing operation in the "female" parent can be done very efficiently because of the following:

1. It is necessary to remove only one flower bud from each plant in order to determine whether the individual is sterile or fertile.
2. Staminate flowers are large and easy to remove.
3. Sterility is readily distinguishable. The pollen sacs of the sterile flower appear black and shrivelled to the naked eye whereas those of the fertile flower appear yellow and plump.
4. Staminate flowers of the female parent open a week or two weeks before pistillate flowers and, hence, ample time is available for roguing before open-sibbing is possible.

The use of such male sterility may reduce considerably the cost of hybrid seed production in *Cucurbita pepo* L.

II. Incidence of Albino Seedlings

Recessive lethal genes are known to exert their catastrophic effect on both plant and animal organisms. In plants, the hereditary failure of certain "albino"



MALE STERILE SQUASH FLOWERS
Figure 5

The sterile bud is on the left in each set. *A* shows a section of a staminate flower before anthesis; *B* a similar bud with its corolla removed.

or "yellow" seedlings to develop chlorophyll under normal conditions for growth is attributed to the action of such genes. Albino seedlings have been found in maize, wheat, barley, tobacco, onion, snapdragon, etc. No albino seedlings have been reported previously in the cucurbits in spite of the fact that cultivated cucurbits were subjected to intensive inbreeding by many workers and for a relatively long period of time. (See Literature references 1, 2, 3, 4, 5, 8, 9, 12, 13, 14, 16, 18.)

Albino seedlings were found by the writer in certain inbred lines of the genus *Cucurbita*. These seedlings do not usually grow beyond the cotyledon stage, perishing a few days after emergence above the ground. Occasionally a yellow seedling may develop chlorophyll and continue to grow to maturity. Such a plant, however, remains a yellowish green and is a poor yielder.

In Table I data are presented on the incidence of albino seedlings in the three species of *Cucurbita* and in Table II similar data are presented for two species of *Cucumis* and one species of *Citrullus*. The data cover 29 varieties of our cultivated cucurbits and a total number of about 80,000 plants observed for lethal genes. The data suggest that albinism may be inherited as a recessive character whose expression is due to the presence of a lethal gene.

Apparently the frequency of occurrence of lethal genes depends on the genus and on the original genetical composition of the open pollinated varieties. The data in Tables I and II show that only sixteen lines out of 302 inbreds were found to segregate for albino seed-

lings. The 16 segregating lines were confined to the genus *Cucurbita;* but, of these, 11 lines came from *Cucurbita maxima* Duch. variety Golden Delicious. It is important to state that all of these segregating lines have been originated in highly variable commercial varieties which were presumably heterogenous and heterozygous at the beginning of the inbreeding experiment. The complete absence of segregating lines in the varieties of *Cucumis sativus, Cucumis melo,* and *Citrullus vulgaris* may have been due to the relative uniformity of the parental varieties; or, perhaps, to the low rate of mutation in these species of low chromosome number. The basic chromosome numbers for *Cucumis sativus, Citrullus vulgaris, Cucumis melo,* and *Cucurbita* are 7, 11, 12 and 20 respectively. The genus *Cucurbita* with a basic number of 20 chromosomes is highly variable genetically and may have a greater number of gene loci, on which chlorophyll development is dependent, than species with basic numbers of 7, 11, or 12. However, mutation rate may depend not merely on the number of genes but on the sensitivity of the same gene loci to the agents of gene mutation.

Consequences of Inbreeding in Cucurbits

There seems to be a general agreement among plant breeders that "loss of vigor" as a result of inbreeding is non-existent in the cultivated cucurbits (see literature references 2, 4, 5, 9, 12, 13, 14, 18). The writer contends that this conclusion is not entirely justified. Many commercial stocks of the cultivated cucurbits are apparently relatively homozygous for major growth factors and therefore do not lose vigor when they are inbred. On the other hand, inbred lines from variable stocks of the same varieties may show some "loss of vigor," expressed in the reduction of the population due to lethal or semilethal genes, in the presence of abnormal types of growth habit which are usually of low yield, and in the decrease in the amount of pollen grains which may adversely affect seed fertility.

Although the cucurbits and maize are known as "open-pollinated" crops they differ in blooming habits and in the vectors responsible for cross pollination. Whereas cross pollination in maize is about 95 per cent, cross pollination in andromonoecious varieties of cucumbers and melons[13] may be as low as 10 per cent. It is about 65 per cent in monoecious varieties of cucumbers;[9] the latter percentage may apply also to the various species of *Cucurbita* and *Citrullus.*

The relatively high percentage of natural selfing may account for the relative stability of our present varieties of cucurbits and for their response to inbreeding. It does not follow, however, that the vigor of cucurbit varieties is retained under all circumstances. Moreover, in our interpretation of heterosis and inbreeding we are too often inclined to make general deductions based on our knowledge of maize manifestation in other genera. The "loss of vigor"—a rather loose term to be sure—may be expressed in certain crops in a manner and in characters which are not readily discernible.

There is need for a more critical analysis of inbreeding in the cucurbits.

Summary

A morphological male sterility is reported which may have considerable practical value in a program of hybrid seed production in *Cucurbita pepo* L. In addition, data are presented on the incidence of recessive lethal genes in inbred lines of *Cucurbita, Cucumis,* and *Citrullus.* Such genes are apparently confined to the genus *Cucurbita.*

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TABLE I. Incidence of lethal recessive genes in various inbred and open-pollinated populations of the Genus *Cucurbita*.

Species	Variety	Number of generations selfed.	No. of lines observed.	Total No. of seedlings observed.	No. of segregating lines.	Total No. of seedlings in segregating lines.	Observed No. of yellow seedlings in segregating lines.	Expected No. of yellow seedlings on the basis of 3:1.
Cucurbita pepo L.	Cocozelle Bush	one	14	558	0	—	—	
" "	Early Golden Summer Crookneck	"	30	1,617	0	—	—	
" "	Fordhook Zucchini	"	27	1,852	2	398	93*	99.50
" "	Giant Crookneck	"	15	713	0	—	—	—
" "	Green Bush Vegetable Marrow	"	9					
" "	Summer Straightneck	"	18	1,196	0	—	—	—
" "	Yankee Hybrid	four	34	1,884	0	—	—	—
" "	Yankee Hybrid	five	10	1,015	1	107	16†	26.75
" "	Table Queen	one	14	605	0	—	—	—
" "	Top of the Market	"	6					
" "	White Bush & Wood's Prolific	"	55	2,433	1	16	4*	4
	Yellow Bush	"	15	453	0	—	—	—
" "	Six commercial varieties	O.P.	—	753	0	—	—	—
" *maxima* Duch.	Blue Hubbard	one	1	56	1	56	12*	14
" "	Boston Marrow	"	1	68	0	—	—	—
" "	Golden Delicious	"	48	1,781	11	452	110*	113
" "	Four commercial varieties	O.P.	—	1,424	0	—	—	—
" *moschata* Duch.	Butternut	one	5 lines massed	1,000	0	—	—	—
" "	Five commercial varieties	O.P.	—	1,012	0	—	—	—
1 genus, 3 species	15 distinct varieties	—	302	19,210	16	1,029	235*	257.25

*A good fit.
†A very poor fit perhaps due to late counting and early death of a number of albino seedlings.

TABLE II. Incidence of lethal recessive genes in various inbred populations of the Genera *Cucumis* and *Citrullus*.

Cucumis melo	Tip Top and Bender	One	6	10,742	0	—	—	—
" *sativus*	Abbott & Cobb	Six	8	11,584	0	—	—	—
	Chinese Long	Eight	4	5,823	—	—	4*	—
" "	Heinz Pickling	Five	1	1,378	0	—	—	—
" "	National Pickling	Five	7	10,452	0	—	—	—
Citrullus vulgaris†	Klondike and 7 other varieties	Three to Eight	many	over 20,000	0	—	—	—
2 genera, 3 species	14 varieties		>26	59,979	—	—	4*	—

*No specific line was found to segregate consistently for albino seedlings but occasionally such seedlings are found.
†Data on watermelons are presented here through the courtesy of Dr. D. R. Porter (personal communication); see also Porter.[12]

THE INHERITANCE OF PITTED EAR

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EAR PIT (*fistula auris congenita*) is defined as a condition in which a sinus is present immediately in front of or at the end of the ascending limb of the helix as illustrated in Figure 6. It is a character of considerable scientific interest for a number of problems are associated with it. Pitted ear is a relatively rare trait, found in a few families. If its mode of inheritance can be accurately determined, it may be useful in deciding cases of doubtful paternity, and as a marker in linkage studies.

Literature on pitted ear appeared as early as 1863, when Betz[1] reported a case of fistula located in the mid portion of the descending limb of the helix, that is, in the rolled posterior border of the external ear. The first data on familial incidence of pre-auricular fistulae were probably published by Heusinger[7] in 1864. More recently pitted ear has been studied by Kindred,[11] Whitney,[22] Quelprud,[17] McDonough,[15] Cannon,[4] Huff and Brillhart,[9] and Pipkin and Pipkin.[16] The early literature has been reviewed by Marx.[14] Edmonds and Keeler[6] report sinuses of the lobe, or most dependent part of the external ear. The similarity between congenital sinuses of the lobe and the sinuses which result from perforations for rings is so great that the question of the inheritance of an acquired character has been raised. However, Bland-Sutton[2] has answered it in the negative.

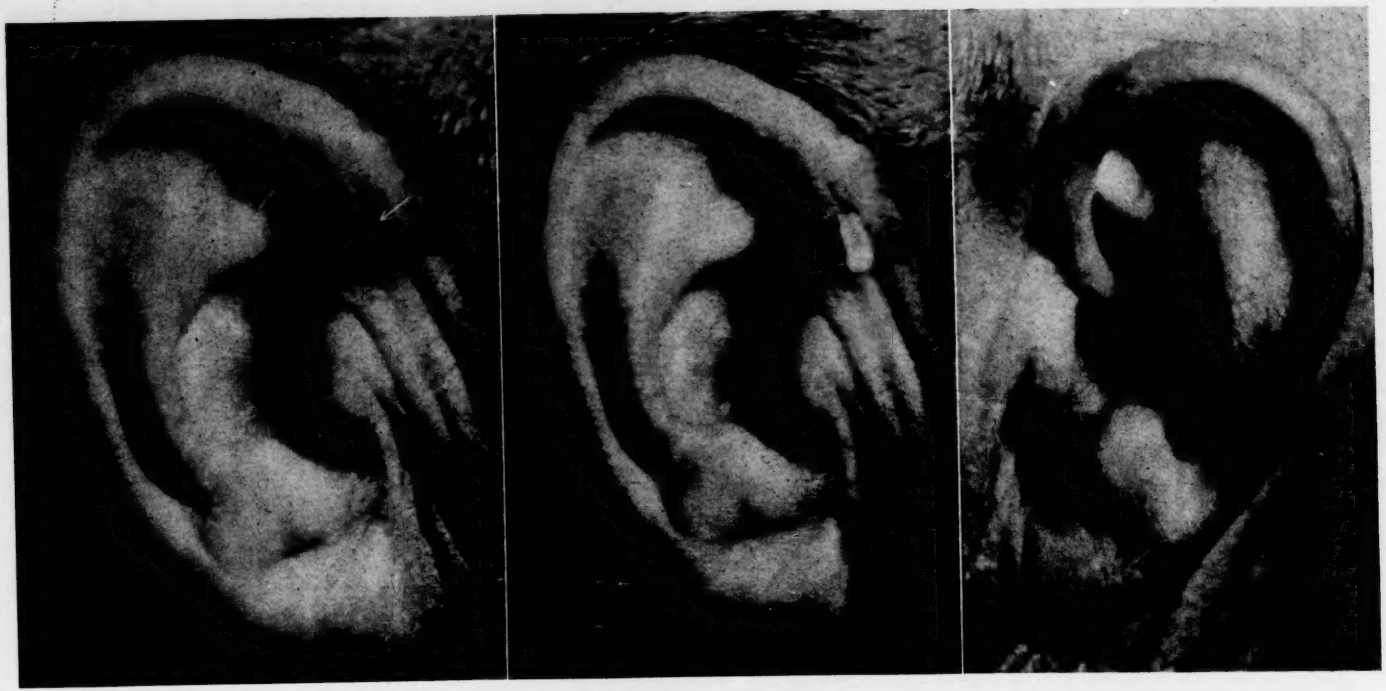
Pedigree Notes

To one of the families of Pedigree *C* (Figure 8) of Dutch ancestry, there is a legend to the effect that the "mark" (pit) as shown in Figure 6 was the result of the ear having been pierced with a sharp instrument. According to this legend, when Irish missionaries attempted to Christianize the pagan Dutch of Holland, they met with much resistance from the Freislanders who would not accept the new faith. Finally the Irish missionaries decided to mark the pagans in some way so that they could be told from the Christians. The story continues that the missionaries took sharp instruments and punctured the auricle of the ear so as to be able to tell the pagan Dutch from the believers. (It is to be inferred that the pagans must have constituted a minority or they would hardly have acceded to this arrangement.) Actually the fistula is on the cheek or at the proximal end of the ascending helix which would make a puncture at the locus of the pit practically impossible. However, the unscientific belief is still held by some of these people that families in which pitted ear is found are descended from the pagan Dutch. One man (III-8 of Pedigree *C*) told the author that he thought he knew of other Dutch families with ear pits, but of the seven pedigrees studied only the families of Pedigree *C* were of known Dutch descent.

The author is greatly indebted to those individuals who generously provided information concerning the pedigrees. More specific acknowledgments would be made if it were not for the fact that the information in some cases was made available with the understanding that no names would be revealed. However, it does not betray any confidence to express most sincere appreciation to Doctor George C. Huff and to Miss Winifred Brillhart who contributed the data for pedigrees *E*, *F*, and *G*.

The seven pedigrees of this study are presented in Figures 7 to 10. Notes regarding them are given in the legends. In addition we are reporting further

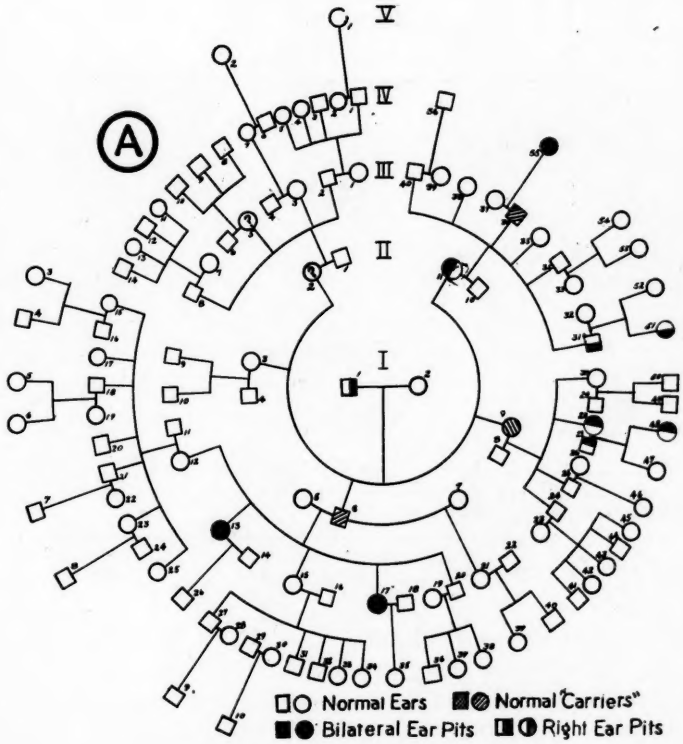
*Contribution from the Science Laboratories of Coe College No. 17 N.S. Aided by an academy grant from the American Association for the Advancement of Science through the Iowa Academy of Science.



UNILATERAL EAR PITS

Figure 6

A—The right ear of III-8 in Pedigree C. Note the pit, indicated by arrow, at the base of the ascending limb of the helix. *B*—A match has been inserted in the pit to give some idea of its size. *C*—The left ear of the same individual. Observe that no pit occurs in this ear; in other words, the expression of the trait in this individual is unilateral.



INHERITANCE OF PITTED EAR

Figure 7

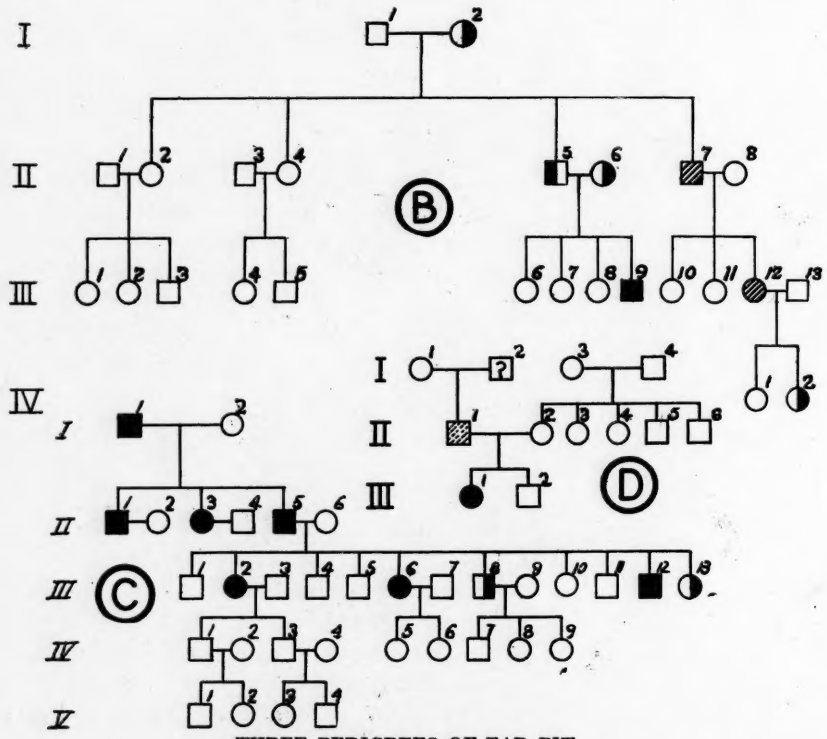
Males are symbolized by squares, females by circles. The black half of the split symbols shows the side on which ear pit occurred. The "carriers" do not themselves have ear pits but have descendants who do. The question marks indicate those about whom information was not available. This pedigree consists of 119 individuals, including, as is customary, all those marrying into the family. Progenies having at least one parent showing the defect totalled seventeen, of whom only four showed pitted ear. One affected woman (III-28) married an unrelated man also having a pitted right ear.

details regarding two of the pedigrees.

Pedigree A

The condition of II-2, who is dead, is unknown. If she possessed pitted ears her children did not know about it, but some other members of the family who have this trait believe that she also had it. III-5 is also dead and no information was available regarding her ears.

The diameters of the pits range from approximately the size of a pin head to about twice that size. The pits also vary considerably in depth; III-17's are about a sixteenth of an inch deep while III-13's are about a half inch deep. The pits of III-13 caused a great deal of pain by becoming inflamed and discharging pus at times, and finally developed into pathological fistulas which were surgically removed. III-28 also at times suffered severe pain from her pit which occasionally discharged a white exudate. Aside from these cases, the pits never caused any trouble. Pit in the ear, un-



THREE PEDIGREES OF EAR PIT

Figure 8

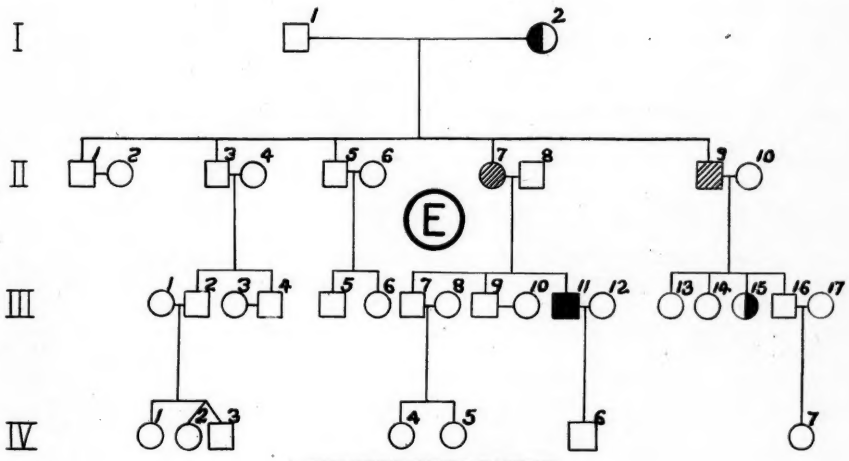
The first generation in pedigree *B* came from England. The families in this pedigree are a mixture of Welsh, Scotch, Irish, German, and Czech. For example, IV-2 is one-fourth Czech, one-fourth Welsh, one-fourth Scotch-Irish, and one-fourth German, which is good evidence that pitted ear is not restricted to one nationality. The occurrence of such a rare character in both husband and wife (B-II—5 and 6) is interesting. The fact that only one child is affected (III-9) is good evidence that ear pit is not a recessive character.

Pedigree *C* shows the highest incidence of pitted ear in the first three generations found in any of the pedigrees here reported. In this pedigree parents, at least one of whom showed the trait, had twenty children, eight of them with pits. II-3 and II-4 are known to have children but no data were available for they live in a foreign country now under the control of Hitler.

Only one person in pedigree *D* is known as possessing pits. III-1 is a young woman eighteen years old; the pits are small and they have never caused any trouble. I-2 is dead and nothing could be learned about the condition of his ears.

less pathological, is considered a neutral trait, that is, one which is neither detrimental nor beneficial. In fact, some families consider it a distinguishing mark of which they are proud. This attitude makes it easier to obtain data on the character than would otherwise be the case.

The occurrence of such an unusual trait in both husband and wife (III-27 and III-28) is interesting. III-27 did not know of anyone else in his family with pitted ear, but he might have had affected relatives for many of them were not well known to him. It will be noted that both parents had the pit only in the right ear and IV-48, their daughter, is similarly affected. In fact, throughout this pedigree, wherever the character is expressed unilaterally and is passed di-



A PIT-CONSCIOUS FAMILY

Figure 9

In pedigree *E* none of the six children of the three affected individuals had ear pits, but two grandchildren of the initial case were affected.

rectly from parent to child, it always appears on the same side in the offspring as in the parent.

Pedigree B

Parents of whom at least one showed the trait, had eight children: two of them exhibited pitted ear. Again it will be observed that husband and wife (II-5 and II-6) have unilateral ear pits but on opposite ears; and their only affected child (III-9) exhibits the trait bilaterally. The fact that III-6, III-7, and III-8 do not show pitted ear indicates that the character is not a simple recessive. The trait as to location and form is the same as reported in Pedigree *A*.

Discussion

The location of the fistulae in the pedigree of this study varies but little from that illustrated in Figure 6. There is, however, greater variation in diameter and depth. Some are pathological but the great majority are harmless. The embryogeny of ear fistulae is uncertain. Betz[1] interprets such pits to be abortive accessory auditory canals, derived from the first branchial cleft. Bland-Sutton[2] considers the fistulae of the lobe due to the persistence of a gap between tubercles numbered 1, 5, and 6 by His.[8] On the other hand, Whitney[22] considers the prehelicine pit a result of lack of fusion of tubercles 2 and 3. But Streeter's[21] studies of human ear development lead to the conclusion that the tubercles are transitory and incidental rather than fundamental in the development of the auricle. Therefore, on the basis of his investigations, it seems unlikely that ear pits result from the persistence of rudimentary tubercles.

Some published pedigrees have shown the inheritance of the fistulae to follow a simple dominant inheritance. Pedigree *C* is in keeping with such a hypothesis. However, in pedigrees *A*, *B*, *E*, *F*, and *G*, it would appear from the fact that the trait skips generations that the gene does not always come to expression in the heterozygous condition. This suggests an irregularity in expression of dominance as has been noted in other pedigrees by Kindred,[11] Whitney,[22] Edmonds and Keeler,[6] Cannon,[4] and McDonough.[15]

There is no way to prove that the gene for ear pits in Pedigree *C* is identical

with that in the pedigrees exhibiting less regular dominant inheritance. To the author it seems more logical to assume that the complete penetrance in this pedigree is due to different genetic modifiers, rather than to a different ear-pit gene. Although the author is inclined to favor this interpretation, nevertheless, it is realized that the final answer to this problem will come from a subsequent study of additional generations and individuals in Pedigree *C* which it is hoped will be made.

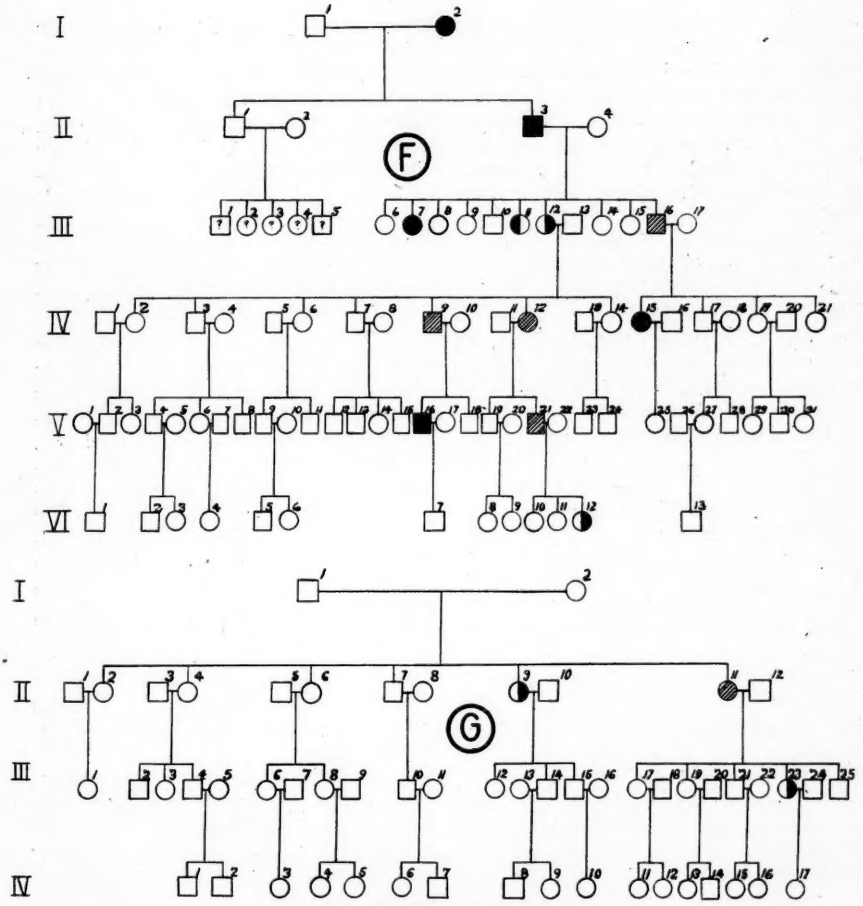
The evidence from the pedigrees as a whole is conclusive (assuming the same gene is involved in all of them) that ear pit is not due to a recessive gene. Considering the rarity of the trait, a multiple factor explanation can be excluded, for the continued outcrossing to the normal should result in the disappearance of the character. If the genes involved were so widely distributed in the population that the skipping of generations could be accounted for by a chance meeting of widely distributed complimentary genes, then it is highly improbable that there would be so few ear pits in the general population. Hence the postulate that a single major gene is involved seems most probable.

On the whole, the data here presented lead to the conclusion that the gene determining pitted ear is irregularly dominant in its expression, the heterozygote frequently being phenotypically normal. All matings except two (which are here excluded) involving persons with ear pits were of affected individuals and normal. These produced a total of 71 children, of whom 16 possessed ear pits. This is a ratio of approximately 1:4. This is in poor agreement with the expected ratio of 1:1 if the trait were a simple Mendelian dominant and the assumption were made that those showing the trait were heterozygous. This latter assumption seems reasonable to make in view of the rarity of pitted ear. The ratio of 1:4 in the families with ear pits might be accounted for by assuming that fistula auris congenita is an irregular dominant, with the heterozygote frequently normal.

Further analysis shows that there are 13 "proved carriers" (cross-hatched in the pedigree charts) as evidenced by pits in subsequent generations. This seems a fairly safe assumption for other explanations for pits in the progenies of "proved carriers" would involve mutations or introduction of the pit gene by outcrossing. These possibilities seem unlikely in view of the rarity of the character. Excluding individuals exhibiting ear pits in the first generation, there are 111 persons who are the offspring of affected individuals or of "proved carriers" from heterozygous matings. From these heterozygous matings there are 29 affected, 11 "proved carriers," and 71 normal, totaling 111. The expected ratio with complete penetrance would be 55.5 normal : 55.5 affected, but the actual ratio is 82:29 or a penetrance of approximately 52 per cent.

We assume that the excess normals above expectation (and within the limits of sampling error) are genotypically heterozygous pitted. On account of the outcropping of affected individuals in later generations, we have evidence that 13 of these normal individuals (cross-hatched in the pedigree) actually carry the ear-pit gene. This strengthens the theory that lack of penetrance, conditioned either by environmental or genetic modifiers, accounts for the observed deficiency of pitted individuals.

Lack of conclusive evidence makes it possible only to speculate on the cause of irregular dominance in the case of pitted ear. Why do some genes vary in their expression? Intrauterine environment at the time of gene action might cause the observed variability. It has been postulated that the variable effect of a gene is due to an unstable condition of the gene. Presumably this might refer either to germinal or somatic tissue. Or a gene may be transmitted unmodified to the offspring, but its phenotypic expression may be altered through the influence of other genes which may become associated with it in consequence of outcrosses. Variability in the degree of expression of a gene is not a rare thing. The difference between variability in expression and total absence is one



MANY SKIPPED GENERATIONS

Figure 10

Pedigree *F* covers a period of time from 1786 to 1941. The families are of Scotch and English descent. Families of this pedigree were proudly conscious of the "family mark." The first question asked the parents at the birth of a child is, "Does it have the family mark?" The pit is always located near the place where the ascending limb of the helix joins the head. Since the second generation, reliable information indicates that there has been no irritation or secretion in the pits. And according to family tradition this was also true for the first and second generations. In this pedigree parents at least one of whom showed the defect, had twenty children of whom only four showed pits.

There are fifty-six individuals in pedigree *G*, but only two have pits—an aunt and her niece. Both are affected only in the right ear. The two affected women had four children, all with normal ears. It would seem a fair assumption that the gene for ear pits is present in I-1 or I-2. Hence we would expect it to come to expression in some of the families on the left side of the pedigree chart, but no cases could be located among these descendants.

of degree. Hence modifying genes might prevent even a so-called dominant gene from coming to expression. Keeler[10] gives a striking instance of varied expressivity of a gene for diabetes, quite similar to the situation here reported.

Of the 38 individuals possessing fistulae, 13 were males and 25 were females. Therefore, it would appear that the character is not influenced by sex. It will also be noted that the trait may be either bilateral or unilateral in expression, and, in the latter case, it may appear on right or left sides. Pipkin and Pipkin[16] summarized the location of ear pits in the pedigree of several authors. Their table shows 30 individuals with pits in both right and left ears, 12 in right ear only and 28 in left ear only. The ratio of right ear pits to left ear pits is 12:28. Edmonds and Keeler[6] suggest from the data of their pedigrees that the greater incidence of left side is due to a lower metabolic gradient on that side. Stannus,[18] on the contrary, found right ear pits with a higher frequency in the African tribes he studied. He found 50 bilateral ear pits, 132 right ear pits, and 110 left ear pits among a total of 6,491 individuals observed.

In our pedigrees the ratio is 16 bilateral ear pits to 16 right ear pits to 6 left ear pits. In contrast with the data of Stannus,[18] these data are collected from white families. These data suggest that no particular principle of development favors the presence of ear pits on the left side over the right side.

The unilateral expression of ear fistulae raises the interesting biological problem of asymmetries. Brauns,[3] Quelprud,[17] and McDonough[15] found that in instances of one-egg twins, only one member of each of six pairs possessed a fistula. It is assumed that the lack of concordance of the anomaly in identical twins is caused by the same factors operating to produce asymmetrical expression in individuals. If this assumption is correct, lack of concordance of pit in one-egg twins suggests that asymmetrical expression of the character is due to non-genetic factors, and these factors may account for the varied expressivity of the gene in our pedigrees.

However, Little[13] has demonstrated by selection experiments that the right-left incidence of inherited foot abnormalities of mice is influenced by genetic modifiers. Thus there is evidence that certain types of asymmetries have a genetic basis. But the consensus of opinion among students of this problem seems to favor a non-genetic explanation of a unilateral appearance of a character. Landauer[12] in research on hereditary cross-beak in chickens found the bending of the beak toward the right side is much more frequent than bending toward the left side, but his studies furnish no evidence for the existence of genetic factors influencing the frequency of the different asymmetry types.

The possibility of a somatic mutation producing the unilateral ear pit seems remote for such genic instability does not appear likely. However, this explanation cannot be completely ruled out.

Stiles[19] pointed out that aphids presumably of the same genotype could be changed from winged to wingless, or vice versa by modification of the environment. In affecting these transitions many aphids intermediate between winged and wingless were produced. Among these intermediate aphids were some with one wing fairly well-developed while the other was represented only by a primordium. This may be cited as a case in which asymmetry is due to non-genetic factors.

Danforth[5] says that "so far as the evidence goes, there is no indication that the unilateral appearance of a variation differs genetically from the bilateral," then explains, "the sides of the body and the sex of the individual act as definite influences in favoring or tending to inhibit the appearance of various traits represented in the germplasm." The answer to the problem of asymmetry lies in the discovery of the nature of the influences mentioned by Danforth.

Professor H. H. Newman states in a letter that asymmetries are due to heterogonic growth of bilateral structures, and as a rule the left side grows faster and, therefore, has a higher metabolic rate. Then he says, "My idea with regard to unilateral expression of hereditary characters such as ear pits, for example, is that at the critical moment of their formation the metabolic rate of the right and left primordia differ in such a way

as to favor the appearance of the character on one side or the other. Sometimes an inherited character might fail to be expressed on either side, or it may appear on both sides, all depending on whether or not the metabolic rate favorable to its appearance is present at the critical time."

The causes for asymmetries will be found in the peculiarities of development. This is suggested by the fact that asymmetries have been produced experimentally. Stiles and Watterson[20] found that jarring developing chick embryos caused asymmetries. Among the many asymmetries appearing, it is most interesting to note that in some embryos only one ear developed. It is postulated that a reduced oxygen supply to the tissues of the jarred embryos was the physiological cause of the asymmetries. Thus it seems likely that asymmetries of development furnish the physiological substratum for teratological asymmetries if normal development is disturbed. But critical evidence is needed to discover whether such developmental factors are responsible for the occurrence and distribution of asymmetries generally.

Our knowledge of these developmental factors is at present too vague to give a completely satisfactory explanation for the origin of asymmetries such as those observed in the pedigrees here reported. The author believes that there is a reasonably good possibility that these deviations may trace back to inequalities of bilateral growth and differentiation, possibly originating from slight asymmetries of circulation in the early stages of embryogeny.

Summary

1. There were 370 individuals in this study of whom 38 possessed ear pits.

2. Ear pit is considered a neutral trait except in rare cases where it is pathological.

3. An analysis of the pedigrees makes it appear that pitted ear is an irregular dominant with a reduced penetrance of approximately 52 per cent and slightly variable expressivity.

4. Literature relevant to the development of ear fistulae is reviewed.

5. Pitted ear was unilateral in expression in 22 of the 38 individuals showing the trait. The biological basis of asymmetries is discussed.

6. Some explanations for irregular dominance are considered.

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FURTHER DATA ON THE INHERITANCE OF LINT COLOR

In the Old World Cultivated Cottons

R. A. SILOW*

IN a recent paper[1] the results of a comprehensive survey of the extensive range of variation in lint color in the cultivated Old World cottons were presented. It was found that three main color loci were concerned, and a variable complex of minor genes. At Lc_1 only one colored allele, a khaki, in addition to white, was identified. At Lc_2 a multiple series of four alleles was recorded—khaki, medium brown, light brown, and white. At Lc_3 again only a single colored member, in this case a light brown, was found in addition to white. Their inter-relationships are shown in Table I.

Table 1. Interrelationship of alleles and loci controlling lint color in the Old World cultivated cottons.

Khaki	Lc_1^K	Lc_2^K	.....	Lc_3^K
Medium brown	.....	Lc_2^M	.....	.....
Light brown	.....	Lc_2^B	Lc_3^B	.....
Very light brown	.....	Lc_2^V	.....	.....
White	lc_1	lc_2	lc_3	lc_4

N.B. Lc_2^V and Lc_1^K are discussed in the present paper.

The expression of the color genes, in particular that of the light browns, was very much dependent upon the genotypic background. In general, in both colored and white-linted strains, the minor genotype of *Gossypium arboreum* was intensifying, while that of *G. herbaceum* was strongly suppressing. It is the purpose of this note to report the results of further investigations which were undertaken to elucidate a few points of interest which were still outstanding when the earlier paper was prepared for publication. Familiarity with the details of the main paper will be assumed, and these findings presented only in outline.

Linkages involving each of the three main loci were recognized, but that concerning Lc_3 and a corolla color locus, Y_a, was only established on the basis of coupling data in a backcross segregation involving two of the lint color loci. The crossover value found was 24%. Repulsion data in backcross families involving only the one lint color locus have now become available (in synthesized material of interspecific origin, using the heterozygote as female):

$Y_a\ Lc_3^B$	$Y_a\ lc_3$	$y_a\ Lc_3^B$	$y_a\ lc_3$	Total
49	85	96	34	264

This material shows 31.5 per cent crossing-over.

A survey of twenty of the twenty-four colored strains in the *arboreum* type collection at the Cotton Research Station, Trinidad, showed that most of the occurrences of color were referable to alleles at the Lc_1 or Lc_2 loci. In addition, one of the remaining four strains, ANBB 15 (Rangamati, a race *burmanicum* representative from the *cernuum* tract) may now be classified as Lc_1^K (Table II).

One of the twenty strains previously tested, ANBB 10, a Bengal strain of race *burmanicum*, was shown to carry a light brown gene, but the location of this gene was not determined. It may now be referred to the Lc_2 locus (Table II).

In three of the twenty strains examined it was established that a color gene was concerned which was not located at Lc_1 or Lc_2; this gene was not tested against Lc_3, which at that time had only been identified in *herbaceum* material. One of the three strains was no longer available, but the remaining two have now been tested:

(i) ANI 10 is an extremely dark-brown-linted strain from Persia. It was previously

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shown to carry Lc_1^K and Lc_2^B. A derivative carrying the third gene, crossed with Lc_3^B and backcrossed to white, gave 17 progeny all light brown, indicating that this strain also carries Lc_3^B. At present this is the only authenticated occurrence of a color gene at this locus in *arboreum*. It may be of some significance that this strain is from the northwestern limits of the range of *arboreum*, where this species is uncommon, but *herbaceum* is widely cultivated.

(ii) ANCB 10 is a khaki-linted strain from Burma, more like the standard Lc_1^K than the slightly reddish Lc_4^K. Its single brown gene was previously shown to be independent of Lc_1, (backcross involving Lc_1^K giving 55 khaki:20 white) and of Lc_3 (in not being linked with H_a—F_2 segregation 45 H_aLc:17 H_alc:3 h_aLc:2 h_alc). It has now been crossed with Lc_3^B, and the backcross to white gave 27 khaki:11 light brown:7 white. It evidently carries a khaki gene situated at a fourth main lint-color locus, of the existence of which this is the first indication. It may be noted that in the Bengal-Burma centre of variability for the species *arboreum*, khaki alleles at Lc_1 Lc_3 and Lc_4 have now been recorded, and a light brown at Lc_2.

In the main publication it was inferred that the khaki allele at Lc_1 was not susceptible to displacement in expression by the modifier distinction which exists between *arboreum* and *herbaceum*. This conclusion, which was then based only on crosses involving *arboreum* Lc_1^K and *herbaceum* strains carrying a suppressed brown at Lc_2, has now been substantiated by examination of an F_2 involving a pure-white linted *herbaceum* strain (H2, Iraq), in which Lc_1^K segregates graded at the same consistent level as in intra-*arboreum* crosses. In a comparable cross involving a khaki allele at Lc_3 there was some indication of a slight effect of modifiers introduced from *herbaceum* on this allele, but nothing comparable in degree with their effect on the lower light brown allele at this locus.

The Lc_2 locus is the only one of the four main lint color loci at which multiple allelomorphism has been found. Some further information on this situation is now available. The duplicate light brown genes Lc_2^B and Lc_3^B were originally derived from a dark brown-linted *herbaceum* strain which was shown to be at much the same high modifier level as the majority of *arboreum* types. Homozygous lines carrying only Lc_2^B derived from this *herbaceum* strain were found to grade at the same level as an *arboreum* strain (ANCC 62) which carries an allele at this locus. ANCC 62 therefore carries the same allele. These introductory remarks are necessary because at the time when it was found that further investigation of the multiple allelomorphism situation was required, suitable material was at hand in a h_a Lc_2^B cross-over segregate in which the latter gene was derived from ANCC 62. In view of the close linkage between h_a and Lc_2, the incorporation of segregation at the former locus greatly facilitates the recognition of distinct color alleles at the latter.

Indirect evidence had suggested that in those very-near-white strains of *G. herbaceum* which carried a strongly-suppressing lint color minor genotype, the main gene concerned at Lc_2 was the light brown allele, Lc_2^B. To confirm this, the very-near-white *G. herbaceum* var. *typicum* strain H10(H_a) was crossed with the h_a Lc_2^B derivative of ANCC 62, and with a lintless strain carrying Lc_2^K. Both F_1s were backcrossed to h_a lc_2. In the first progeny H_a segregates, carrying the linked color allele from H10, graded at the same level as the h_a segregates in the same family. In the second progeny h_a segregates, carrying Lc_2^K, were considerably darker (Table III). It may be concluded that H10 does carry Lc_2^B, as previously suggested. A similar test confirmed that the *G. herbaceum* var. *africanum* strain O14 with very light brown lint also carries the same allele.

In the main publication it was also shown that in two Peninsular Indian strains belonging to *G. arboreum* race *indicum* which had colored lint, a main allele of low potency at the Lc_2 locus was concerned, though its position in the multiple allelomorph series was not

TABLE II.—Allelism tests—backcross data

	Colored	White
(ANBB 15 × Lc_1^K) × white	52	0
× Lc_2^K) × white	37	9
× Lc_3^B) × white	29	14
(ANBB 10 × Lc_1^K) × white	52	23
× Lc_2^K) × white	49	0
× Lc_3^B) × white	35	11

more precisely determined. It was further suggested that these two strains were of low modifier level relative to the majority of other *arboreum* strains in other geographic races of the species. One of them was ATI 5, a strain with light brown lint of the type known commercially as "Red" Cocanadas. This strain grades somewhat lower than is usual for Lc_2^B on most *arboreum* backgrounds (1.5 as against 1.8). ATI 5 has now been crossed with the h_a line carrying the standard Lc_2^B from ANCC 62. Backcross data (Table III) show that the main color allele concerned is a lower allele than Lc_2^B (symbolised as Lc_2^V in Table I), and since the color of the strain itself is so near that exhibited by Lc_2^B on the common *arboreum* background, the modifier level of ATI 5 must be somewhat higher than usual. The low level of earlier grading which was previously interpreted as suggesting a low modifier level in the parental strain was evidently largely due to the low potency of the main allele. From similar evidence, also presented in Table 3, it has now become apparent that the second colored Peninsular Indian strain, the wild ATI 1, which was of very light grade (0.5), also carries an allele lower than Lc_2^B, possibly even lower than that carried by ATI 5.

Some discussion has already been based[2] on the earlier indications that the *indicum* section of *arboreum* was at a low level relative to the bulk of this species. Though the evidence previously brought forward is incontrovertible that some white-linted strains of this race, which lack a main color allele, are at a low modifier level, the situation now demonstrated for the two colored strains shows that this is not always so. Nevertheless this situation in no way affects the arguments which were developed in that paper. The significant feature of race *indicum* appears to be its extreme variability in minor lint color genotype. That such is the situation is borne out by the great variability in tinge of commercial Red Cocanadas, and the difficulty experienced by breeders in establishing lines of constant intensity. Whether there is a general tendency in this race for colored strains to be high and for white and near-white strains to be low in their content of modifiers is worth further investigation. Such a highly variable genotypic background as exists in this race offers ideal opportunity for the development of the high level which characterises the greater part of *arboreum*, on the basis of the main gene switch mechanism postulated[2] for the maintenance of the extreme color alternatives in a single crop population in the early days of cotton domestication.

Summary

Genotypic analysis of the strains with colored lint in the Trinidad type collection of cultivated Old World cottons has been continued. A fourth main locus has been recognized in a strain of *G. arboreum;* further multiple allelomorphism at the Lc_2 locus has been demonstrated; and considerable modifier variability has been found in *G. arboreum* race *indicum*.

Literature Cited

1. Silow, R. A. *J. Genet.* 46:78. 1944.
2. ————. *J. Genet.* 46:62. 1944.

TABLE III. Allelic identity tests—backcross data

		Lint-color grades					
		1.8	1.0	0.8	0.5	0.3-0.2	0.1
(H10 × $h_aLc_2^B$) × h_alc_2	H_a	—		12		4	—
	h_a	—		18		3	—
(H10 × $h_aLc_2^K$) × h_alc_2	H_a	1*		17		10	—
	h_a	32		—		—	—
(ATI 5 × $h_aLc_2^B$) × h_alc_2	H_a	—	—	4	21	5	2
	h_a	—		32	—	—	—
(ATI 1 × $h_aLc_2^B$) × h_alc_2	H_a	—		—	—	27	
	h_a	—		40	—	1*	

*Cross-overs.

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